

Lesman et al., <http://www.jcb.org/cgi/content/full/jcb.201309029/DC1>

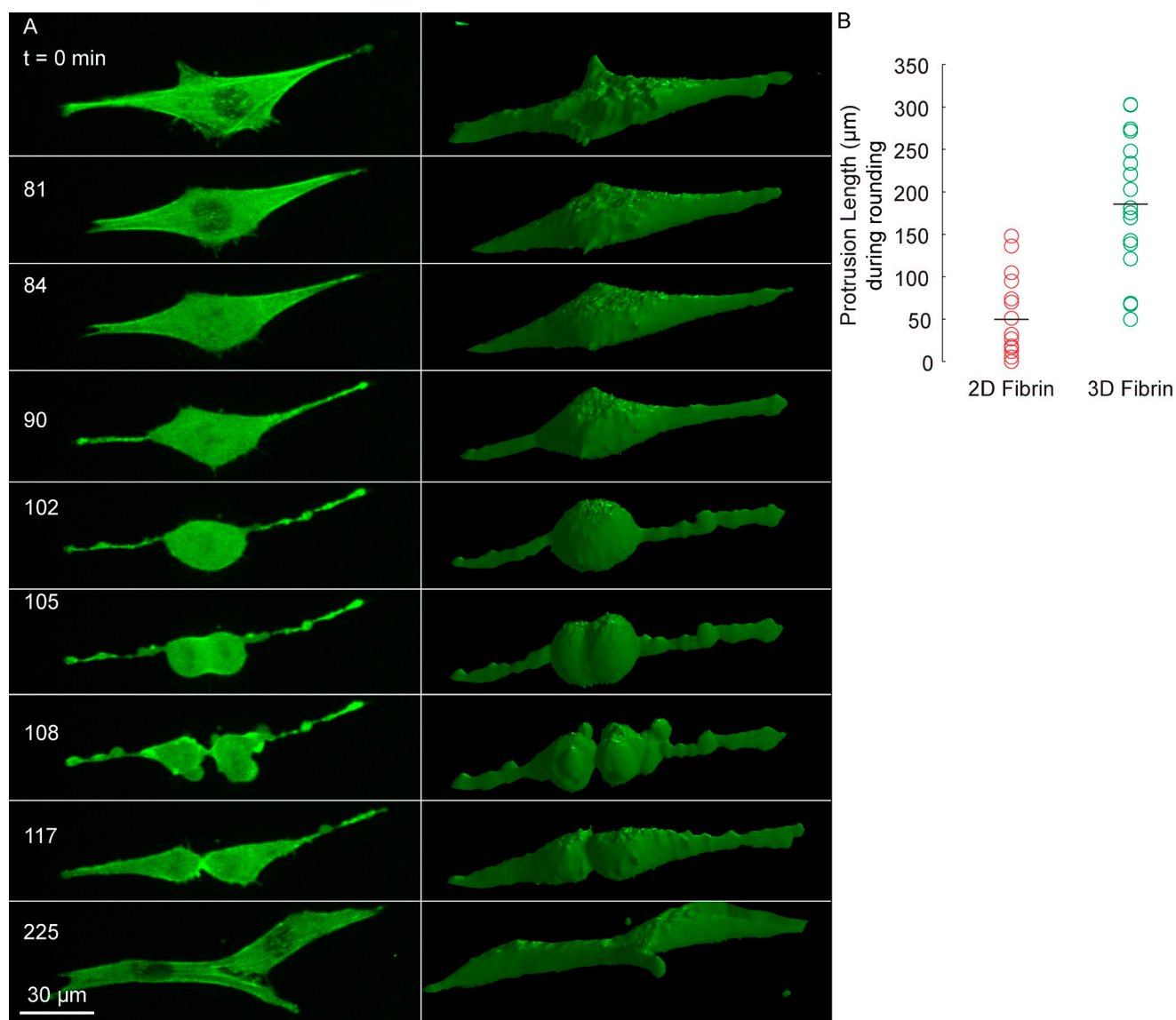


Figure S1. **Interaction of dividing fibroblasts with fibrin matrices.** (A) Maximum intensity projections and 3D isosurface renderings of the dividing fibroblast shown in Fig. 1. (B) Protrusion lengths during rounding for cells plated on a fibrin substrate ("2D fibrin,"  $n = 16$ ) or embedded within a fibrin matrix ("3D fibrin,"  $n = 18$ ).

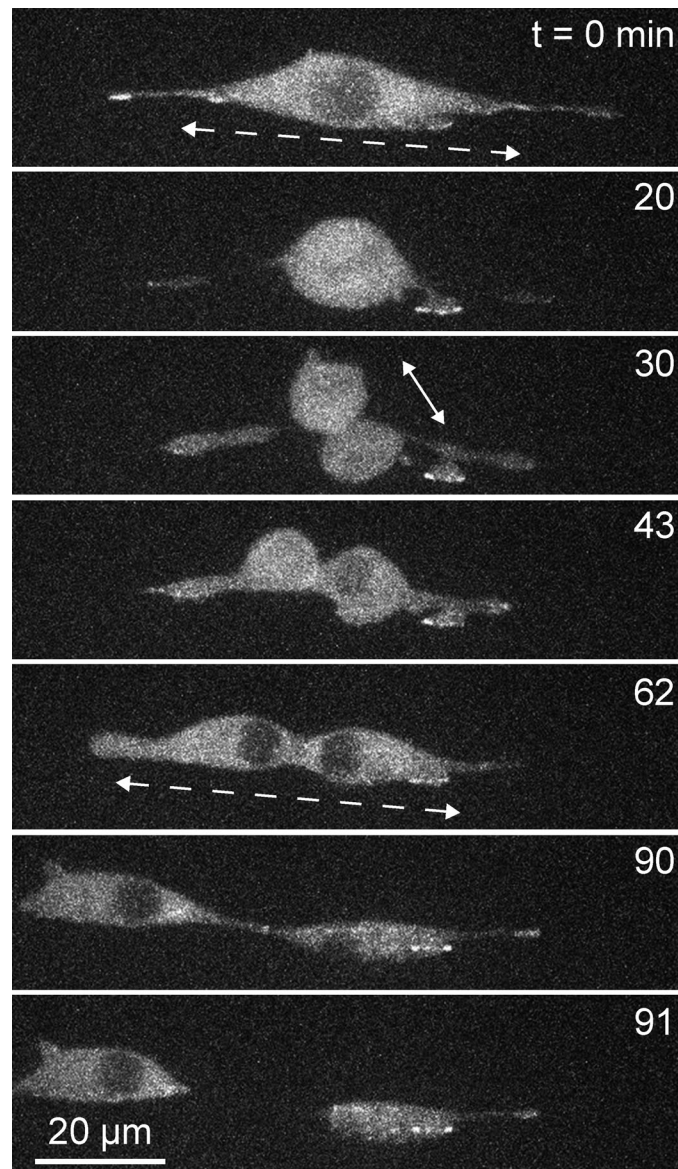


Figure S2. **Daughter cells respread and separate from one another along the long axis of the mitotic protrusions.** Even in events where the division axis ( $t = 30$  min, solid arrow) was not aligned with the direction of the protrusions (broken arrow), the daughter cells shifted their orientation and spread along the protrusions ( $t = 43$ – $62$  min).

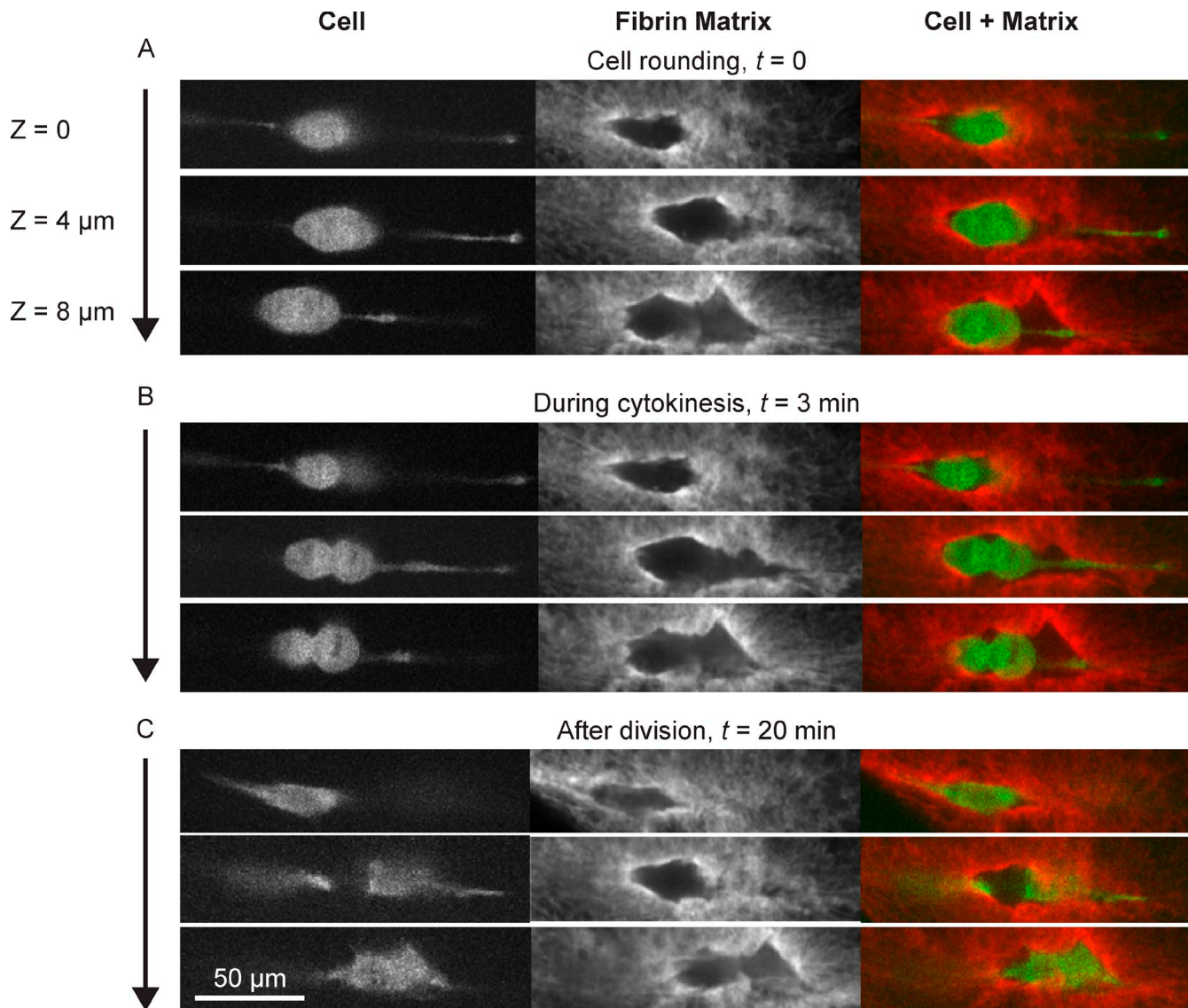
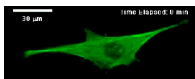
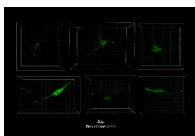


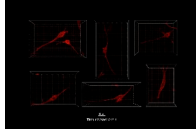
Figure S3. **Direct visualization of cell-matrix interactions during division.** (A–C) Fibroblast (gray in the left panel, green on the right panel) embedded in a fibrin matrix (gray in the middle panel, red on the right). Each image shows one confocal plane as the cell rounds to a sphere (A), during cytokinesis (B), and after division (C). Although the protrusion tips are well embedded in matrix, voids around the cell body indicate that the body disengages from the matrix during division. After cytokinesis, the daughter cells take on the shape of the matrix void (C).



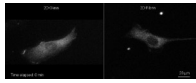
Video 1. **Maximum intensity projections of a 3D image of a 3T3 fibroblast (expressing actin-GFP, shown in Fig. 1) before, during, and after division in a 3D fibrin matrix.** Images were acquired with time-lapse confocal microscopy (Swept Field; Nikon). Frames were taken every 3 min for 230 min. The initially spread cell rounds into a sphere while maintaining long, thin protrusions during division. The cell divides and the daughter cells respread along the protrusions.



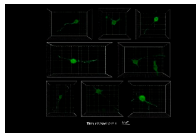
Video 2. **Representative maximum intensity projections of dividing cells embedded in 3D fibrin matrices (3T3 actin-GFP fibroblast cells).** The movies are synchronized such that each shows 75 min before and after cytokinesis. Images were acquired with time-lapse confocal (Swept Field; Nikon) or two-photon (LSM 710; Carl Zeiss) microscopy. Frames were taken every 5 min.



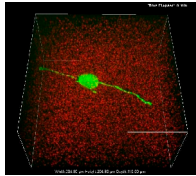
Video 3. **Representative maximum intensity projections of dividing 3T3 fibroblast cells stably expressing LifeAct-mRuby.** The movies are synchronized such that each shows 75 min before and after cytokinesis. All of the cells maintain long, thin protrusions throughout the division process. Images were acquired with time-lapse confocal microscopy (LSM 710; Carl Zeiss). Frames were taken every 5 min.



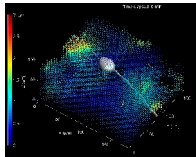
Video 4. **Maximum intensity projections of a 3T3 actin-GFP cell dividing on an uncoated glass substrate (on the left) or on top of a thick (~200  $\mu\text{m}$ ) fibrin gel (on the right), collected with a confocal microscope (Swept Field; Nikon) every 5 min for 150 min.** The movie is from the experiments shown in Fig. 2 (A and B). The cell cultured on glass retracts its long protrusions completely during rounding. The cell plated on the fibrin gel partially retracts its long protrusions during rounding.



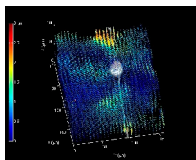
Video 5. **Examples of dividing 3T3 fibroblast cells (labeled with actin-GFP) embedded in 3D fibrin matrices in the presence of blebbistatin (50  $\mu\text{M}$ ).** The movies are synchronized such that each shows 75 min before and after formation of the contractile actin ring. Images were acquired with time-lapse two-photon microscopy (LSM 710; Carl Zeiss). Frames were taken every 5 min.



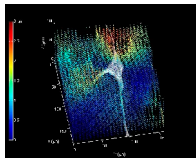
Video 6. **3D maximum intensity projection of fluorescent particles (red; Invitrogen) surrounding a 3T3 dividing fibroblast cell (actin-GFP, green).** The movie is from the same experiment as Fig. 3 (B and C). Frames were collected with a confocal microscope (Swept Field; Nikon) every 30 min.



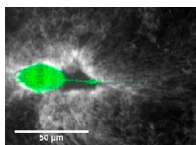
Video 7. **Vector plots of matrix displacements and 3D isosurface renderings of a 3T3 fibroblast cell during and after division.** Frames were taken every 30 min for 570 min with a confocal microscope (Swept Field; Nikon); displacements were computed using DVC and plotted in MATLAB. The dividing cell shown in Fig. 3 applies inward displacements to the matrix at the tips of its protrusions. After division, the displacements increase at the leading edges of new extensions as the daughter cells begin to spread. Displacements decay to zero after blebbistatin is injected into the medium.



Video 8. **Multi-angle view of Fig. 3 C,  $t = 0$ , showing vector plots of matrix displacements during mitotic rounding of a 3T3 fibroblast cell labeled with GFP-actin.** Displacements were computed using DVC. Vector plots and 3D isosurface renderings of the cell were generated using MATLAB.



Video 9. **Multi-angle view of Fig. 3 C,  $t = 210$  min, showing vector plots of matrix displacements around spreading 3T3 fibroblast daughter cells labeled with GFP-actin.** Displacements were computed using DVC. Vector plots and 3D isosurface renderings of the cell were generated using MATLAB.



Video 10. **Time-lapse series of a single confocal plane of a 3T3 dividing fibroblast cell (actin-GFP, green) embedded in a fibrin matrix (labeled with Alexa Fluor 546, gray).** Frames were taken every 3 min for 90 min with a confocal microscope (Swept Field; Nikon).